

**REMARKS**

The Official Action of March 30, 2000 has been carefully considered and reconsideration of the application as amended is respectfully requested.

Applicants note that the restriction requirement has been converted into an election of species. In response to the requirement for an election of species, Applicants hereby elect the species of Group B drawn to an antisense RNA-mediated method for inhibiting trehalase. Claims 35, 37, 38, 42 - 48, 51 - 58, 61 and 62 read upon the elected species. In addition, new claims 63 - 65, introduced by this Amendment, read upon the elected species. New claims 63 - 65 draw support from the specification as filed at, for example, page 2, lines 2 - 5 and Example 8 on page 25.

Notwithstanding that Applicants have made an election in accordance with the requirement in the Official Action, Applicants understand that, upon the allowance of a generic claim, Applicants would be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141.

The claims as amended are free of the informalities noted by the Examiner at page 11 of the Official Action. In particular, claim 42 has been amended to make clear that this claim covers the production within the plant by recombinant DNA methods of a protein that is a chemical inhibitor. Claim 56 has been separated into two claims as courteously suggested by the Examiner. All claims as amended are believed to be sufficiently definite to satisfy the

dictates of 35 USC 112, second paragraph.

The claims have been rejected under 35 USC 112, first paragraph, for alleged lack of enablement. Applicants respectfully traverse this rejection.

The test of enablement is whether one of skill in the art could make or use the claimed invention from the disclosure coupled with information known in the art without undue experimentation (see MPEP Section 2164.01). The factors that must be considered in determining whether any necessary experimentation is “undue” include the following:

- a) The breadth of the claims;
- b) The nature of the invention;
- c) The state of the prior art;
- d) The level of one of ordinary skill in the art;
- e) The level of predictability in the art;
- f) The amount of direction provided by the inventor;
- g) The existence of working examples; and

h) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. *In re Wands*, 858 F. 2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

In the present case, the Examiner has alleged that the Applicants' specification is not enabling for the invention as claimed based upon a review of the following factors: claim breadth, unpredictability, and lack of guidance. Applicants respectfully disagree and submit that an analysis of these factors shows that one of skill in the art could practice the invention as claimed without undue experimentation.

Breadth of the Claims

The claims are respectfully not of the breadth contended by the Examiner. The claims are written in Jepson format so as to separate those elements or steps of the claimed combination that are conventional or known from those elements or steps that constitute that portion of the claimed combination which Applicants consider as the new or improved portion (see 37 CFR 1.75(e)). The application need not teach, and preferably omits, those elements or steps which are known. See, e.g., MPEP Section 2164.01 and cases cited therein. The known elements include plants which produce trehalose either naturally or by transformation thereof (see preamble of claims 35 and 63 and "State of the Art" section on page 1 of the specification).

The invention as claimed takes as a starting point (known) plants that naturally synthesize trehalose or that have been genetically altered to synthesize trehalose. By placing these elements in the preamble of claims 35 and 63, Applicants acknowledge that these elements are known and

that they are not part of the subject matter which Applicants regard as their invention. Accordingly, it is respectfully believed to be irrelevant to the invention as claimed whether the trehalose synthesis "takes place via a single step involving one single enzyme or multiple steps entailing the use of more than one enzyme encoded by more than one gene" or whether "trehalose synthesizing enzymes are the same in all plant species" or if "genes encoding trehalose synthesizing enzymes are the same in all plants". It is further respectfully believed to be irrelevant whether or not optimization of the coding sequence for a TPS gene would be required or if the genetically altered plants can be produced by transformation with a vector comprising a TPS gene from a non *E. coli* source or any other transgene. In any event, Applicants respectfully direct the Examiner to the following references which discuss increasing trehalose content using non *E. coli* TPS.

1. International patent application WO97/42327 (Universidad Nacional Autonoma de Mexico) which concerns a method for increasing trehalose content in organisms, which method involves transformation of the organism with TPS of *Selaginella lepidophylla*; and

2 International patent application WO93/17093 (Oy Alko) which concerns the use of yeast TPS.

There is therefore no reason to believe that TPS from a non *E. coli* source would not be suitable. A person skilled in the art could simply use TPS genes known to be suitable or would use routine experimentation to identify suitable TPS genes.

In short, the claimed invention does not address the synthesis of trehalose as such; it

concerns a method for enhancing the production of trehalose, irrespective of which way the synthesis of trehalose takes place. Thus, for the claimed invention there cannot be a lack of guidance how to conduct trehalose synthesis since this is not part of the claimed improvement. The invention departs from a situation where trehalose synthesis capability is present and covers only how to boost this production by inhibiting trehalase activity.

With respect to the Examiner's remarks concerning the identification and isolation of any gene from any source encoding an enzyme that would confer trehalase-synthesizing ability to a plant (see paragraph 1 on page 6 of the Official Action), Applicants respectfully call the Examiner's attention to the fact that the claims have been amended so that they include only plants which naturally synthesize trehalase. With respect to plants which naturally synthesize trehalase, these are admitted as known (see preamble of claims 35 and 63). Moreover, Applicants respectfully direct the Examiner's attention to page 2, lines 2 - 5 of the specification where it is stated that "In spite of the absence of trehalose as a substrate in most higher plant species, the occurrence of trehalose degrading activity has been reported for a considerable number of higher plant species, including those known to lack trehalose". In other words, it is not necessary for Applicants to provide guidance for plants which naturally synthesize trehalase since those of skill in the art are already aware of such plants. As discussed above, a specification need not include, and preferably omits, that which is known in the art.

In short, the invention as claimed is based on Applicants' unexpected findings that production of trehalose in plant cells can be enhanced by inhibiting the endogenous trehalase activity within the same cells. This inhibition can be achieved by two methods, namely:

- a) by use of a chemical inhibitor which substantially reduces the activity of the endogenous trehalase protein, which chemical inhibitor can be applied exogenously to the plant or produced within the plant by recombinant DNA methods; or
  
- b) by the inhibition of the plants' endogenous trehalase gene by recombinant DNA methods thereby reducing the plants' ability to produce endogenous trehalase. This can be achieved by methods such as sense co-suppression or antisense inhibition.

Therefore, the claimed invention is concerned with inhibition of endogenous trehalase activity in a plant (already containing genetic information for the production of trehalose) such that the plant is able to produce trehalose without suffering the adverse effects of the trehalose being catabolized by the endogenous trehalase. This is the (only) invention for which enablement can be at issue.

Amount of Direction (or Guidance) Provided by the Inventors

The Examiner contends that the specification lacks proper and sufficient disclosure to enable one of skill in the art to practice the invention as claimed. It is respectfully submitted that, in reaching this conclusion, the Examiner has overlooked the extensive guidance that the specification provides. For example, the specification teaches that plants producing trehalose can be used in the method according to the invention to enhance production of trehalose in the plant. Examples 1 and 8 illustrate that, when endogenous trehalase activity is reduced (by chemical inhibition of an endogenous protein or inhibition of the endogenous protein formation respectively) the plants' ability to accumulate trehalose is increased. In other words, the

specification provides actual working examples of the invention in a process for increasing trehalose levels in plants. Moreover, the specification as filed provides examples of chemical trehalase inhibitors (according to (a) above) as described *inter alia* on page 5, line 30 - page 6, line 17 and page 7, lines 20 - 28. Methods for inhibiting production of endogenous trehalase are discussed in the specification as filed *inter alia*, on page 6, line 18 - page 7, line 19 and page 9, lines 7 -12.

In addition, and perhaps more significantly, the specification, including the Examples provides a template that a person of skill in the art can use routinely to test (a) other chemical trehalase inhibitors and (b) other RNA transcripts for their effectiveness in inhibiting expression of endogenous trehalase activity. The significance of this will be discussed next.

#### The Quantity of Experimentation

The quantity of experimentation needed to be performed by one skilled in the art is one factor involved in determining whether “undue experimentation” is required to make and use the claimed invention. *Wands* at page 1404. As discussed in MPEP Section 2164.06, the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.” *Wands* at page 1404. For example, in *United States v. Teletronics, Inc.* 857 F. 2d 778, 8 USPQ 2d 1217 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989), the court reversed the findings of the district court for lack of clear and convincing proof that undue experimentation was needed. The court ruled that since one embodiment (stainless steel electrodes) and the method to determine dose response was set

forth in the specification, the specification was enabling. The question of time and expense of such studies, approximately \$50,000 and 6 - 12 months standing alone, failed to show undue experimentation.

In the present case, even assuming that (as contended by the Examiner) trial-and-error would be required to determine the effectiveness of other trehalase inhibitors in the claimed method, any such experimentation would be routine. As discussed above, the techniques for testing other chemical inhibitors or RNA transcripts are straightforward and are laid out in the specification. Given the routine nature of the experimentation to test other trehalase inhibitors, there is no basis for considering that any experimentation needed would be undue.

#### Predictability

To support the enablement rejection, the Examiner has presented a scholarly explanation of enzyme kinetics and how enzyme inhibition is achieved and the various types of inhibition. These factors are respectfully believed to be at best tangential to the issue under consideration. First, the explanation itself indicates that those of skill in the art (e.g., those having a knowledge of enzyme kinetics) who wish to find other suitable trehalase inhibitors for use in the claimed method would be able to rule out certain classes of enzymes. (Note: the inhibition as claimed is directed to a method comprising the step of inhibiting the endogenous trehalase activity of a plant and not to trehalase inhibitors *per se*. The method does not read on trehalase inhibitors that do not work in the claimed inhibiting step.) More importantly, as discussed above, experimentation with other prospective trehalase inhibitors is acceptable where as here such experimentation is routine.

In any event, the kinetics of enzyme inhibition has no relevance to the question of the enablement of the elected species and the claims reading thereon (see, e.g., claim 63). These claims do not encompass protein-protein interactions.

The Examiner also contends that the introduction of single genes which encode a single enzyme in a metabolic pathway may be insufficient to effect the desired phenotype in transformed plants. In connection with this, the Examiner cites Smith et al (*Nature*; 1998, Vol. 334 (25): 725 - 726) where the desired retardation of fruit softening in tomato plants transformed with antisense polygalacturonase was not seen. However, the introduction of single genes is irrelevant as far as the elected species is concerned.

The Examiner states that "the process of modifying carbohydrate accumulation in transgenic plants is particularly unpredictable" and in connection with this cites Kossmann et al (1995; *Progress in Biotechnology*, Vol. 10). Unpredictability may be the case as far as starch metabolism is concerned since starch metabolism involves more enzymes and is more complex than the pathway leading to trehalose synthesis. However, Applicants respectfully submit that a reference concerning the lack of influence of antisense potato starch accumulation genes on branching or phosphate content of starch; the difficulty inherent in isolating individual starch synthesis enzymes or their corresponding genes; and the lack of correlation between reduction of branching enzyme genes activity and branching of starch in transgenic plants would certainly not lead a person skilled in the art conclude that similar problems would be expected with respect to the claimed invention which involves the less complex trehalose pathway.

Regardless of any alleged unpredictability with non exemplified trehalase inhibitors, it

is respectfully submitted that one of skill in the art can practice the invention as claimed with routine (i.e., not undue) experimentation. Accordingly, it is respectfully submitted that the specification is enabling for the invention as claimed and that the rejection under 35 USC 112, first paragraph should be withdrawn.

Certain of the claims have been rejected under 35 USC 102(b) as allegedly being anticipated by Kendall et al. Applicants respectfully traverse this rejection.

Kendall et al discuss the effects of trehalase degradation in cell suspension cultures using GC-MS analysis. The authors set up cell cultures, added an amount of trehalose and a trehalase inhibitor and then incubated the cultures (note that the cited document does not even hint or suggest that the plant cells themselves contain the genetic information to produce trehalose). The aim of the experiment was to analyze effects of trehalase inhibitors on plant trehalases in a culture medium by adding trehalase inhibitors to the medium. The degree of inhibition was measured by GC-MS of the samples, measuring levels of trehalose in the samples. The results showed that, in some cultures, trehalose added to the culture was not catabolized, indicating the effectiveness of trehalase inhibitors in reducing the ability of plant trehalases to catabolize the added trehalose.

At the time of publication of the cited document, it was not known how trehalose could be produced in plants (see, Kendall et al at page 2527, first column, last sentence "naturally occurring trehalose was not detected in the plant materials in this study"). Therefore, one could not have predicted from the cited document with even a reasonable expectation of success that

inhibition of trehalase activity would result in accumulation of trehalose in plants. Furthermore, at the time of publication of Kendall et al, it was not known if validamycin could enter plant cells and exert its inhibitory properties locally. Application of *in vitro* activity to an *in vivo* situation would also not have been predictable. Further still, it was not known at the time of publication of the cited document if inhibition of trehalase *in vivo* would result in increased trehalose content. A situation involving transgenic production of trehalose in a cell compartment other than the compartment containing the trehalase enzyme could not have been ruled out. Accordingly, one of skill in the art could not have had a reasonable expectation of success in inhibiting trehalase activity by applying validamycin as an inhibitor so as to increase the trehalose content in a plant. The cited document does not show or suggest that inhibition of endogenous trehalase can be used to accumulate trehalose *in vivo*. At most, the cited document teaches that validamycin can be used to block trehalase activity found in the tissue of some plants. Accordingly, it is respectfully submitted that Kendall et al does not destroy the novelty of claims 24, 35, 36, 41, 42, 43 to 44, 47 - 48, 49, 50, 56 and 59 to 60 of the present application.

The Examiner rejects claims 46 - 47 on the grounds that these claims are allegedly anticipated by WO95/01446 in the name of Hoekema et al. The cited document contains no hint or suggestion of a stress-tolerant plant containing about 0.01 % fresh weight of trehalose. The Examiner will therefore appreciate that claims 46 - 47 cannot be anticipated by Hoekema et al.

The Examiner rejects claims 24 - 25, 35, 36, 39 - 50, 56, 59 - 62 as allegedly being obvious over Hoekema et al taken with Kendall et al. Applicants respectfully disagree. First of all, Applicants note that, in support of the enablement rejection (discussed *supra*) the

Examiner has contended: "it is unpredictable if trehalose accumulation would have taken place in genetically altered plants other than those exemplified in the specification . . ." (see Official Action at page 8, first full paragraph). It is respectfully submitted that the Examiner cannot have it both ways; trehalose accumulation could not have been unpredictable for the enablement rejection but predictable for the prior art rejection. (By contrast, Applicants respectfully submit that the guidance in Applicants' specification provides a measure of predictability that was lacking in the prior art.)

In contrast to the Applicants' specification, Hoekema et al only teach a method for providing plants unable to produce trehalose naturally with the ability to produce trehalose. The method according to the cited document comprises introducing into cells of plants a recombinant polynucleotide encoding trehalose phosphate synthase under the control of regulatory elements necessary for expression of the recombinant DNA in plant cells. In contrast, the claimed invention concerns a process for producing trehalose which involves growing or cultivating a plant/part of a plant or plant cells in the presence of a trehalase inhibitor to accumulate trehalose. Hoekema et al do mention at page 13 the possibility of producing trehalose in plants that exhibit trehalase activity and state that "it may be necessary to provide for inhibition of trehalase activity". It is also mentioned that inhibition of trehalase activity can be achieved using an antisense approach. However, describing a possibility that it may be necessary to inhibit trehalase activity to produce trehalose is a far cry from showing that inhibition of trehalase activity is sufficient to accumulate trehalose in plants. This Hoekema et al disclosure is no more than an invitation to experiment (without expectation of success) and would not put a person skilled in the art in possession of the claimed invention. Furthermore, at the time of publication

of Hoekema et al, the belief was that an accumulation of trehalose would cause irreparable damage to a plant (see, for example Plant Phys. 68, pages 1369 - 1374, 1981 (Veluthambi et al)). Therefore, provision of a method for accumulating trehalose in plants without the plant being subjected to the damaging effects of trehalose was unexpected.

Hoekema et al do not show or suggest a process whereby plant cells, a plant or a part thereof is cultivated in the presence of a trehalase inhibitor. Cultivation is defined in the authoritative Collins English Dictionary as the improving or harvesting of crops or plants and the preparation of ground to promote their growth. According to the understanding at the time of the invention, such "cultivation" would not have been possible because of the irreparable damage to a plant which would have been caused by inhibition of trehalase, as taught by Veluthambi et al. Accordingly, Hoekema et al, when read in light of Veluthambi et al, would teach away from the claimed invention.

As discussed above, the Kendall et al reference teaches little more than that trehalase inhibitors can inhibit plant trehalases (it does not show or suggest plant cells capable of producing trehalose). Therefore, even if a skilled person were to combine Kendall et al with Hoekema et al they would still not expect success with the claimed invention. Kendall et al fails to suggest that the trehalase protein remains within the cell of the plant, i.e. that it has endogenous activity within the cell. Furthermore, Kendall et al does not give any indication as to where the trehalase is located in the plant. The document might well lead a skilled person to conclude that the trehalase is exported from the cells since the document describes the addition of trehalose and trehalase inhibitor to the culture. The cited document certainly would not provide a person skilled in the art with a reasonable expectation that the exogenous addition of

a trehalase inhibitor would effect an *in vivo* inhibition of intracellular trehalase activity in plants. As there are no definite teachings from Kendall et al, other than that trehalase inhibitors exist which may inhibit plant trehalases, a person skilled in the art could not use this document, either taken alone or combined with any other document in the art, to arrive at the claimed invention.

In view of the above, all rejections and objections of record are believed to have been successfully traversed and the application is believed to be in allowable form. An early Notice of Allowability is earnestly solicited and is believed to be fully warranted.

Respectfully submitted,

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